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ANNUAL PROGRESS REPORT

Grant#: N00014-92-J-1115

R&T Code: 441d023

PRINCIPAL INVESTIGATOR: Dr. John Lee SpudichINSTITUTION: The University of Texas Medical SchoolGRANT TITLE: Role of Protein Methylation in
Halobacterium halobium PhototaxisREPORTING PERIOD: October 1, 1991 - August 31, 1992AWARD PERIOD: October 1, 1991 - January 31, 1992WITH COST EXTENSION October 1, 1991 - January 31, 1993

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SEP 25 1992

OBJECTIVE: To investigate the role of methyl-accepting proteins in the phototaxis signaling system of *H. halobium* membranes. A carboxylmethylated protein in the membrane, MPP-I (methyl-accepting phototaxis protein I) appears to relay the signal from photoactivated sensory rhodopsin I (SR-I, a visual pigment-like photosensor). Our primary objective is to elucidate the relationship between SR-I and MPP-I.

APPROACH: MPP-I primary structure and other properties are being determined by purification of the protein, tryptic digestion and isolation of fragments for peptide sequencing, and use of sequence-derived oligonucleotide probes to clone the MPP-I-encoding gene.

ACCOMPLISHMENTS (last 12 months):

In earlier work on this project a methylated membrane protein of 97kDa M_r was suggested on the basis of mutant analysis to transduce signals from the phototaxis receptor sensory rhodopsin I to the flagellar motor in *H. halobium* (Spudich et al, Proc. Natl. Acad. Sci. USA 86:7746-7750, 1989). In this period we completed the cloning of the proposed transducer protein gene based on partial protein sequences from the isolated protein, the complete gene sequence and analysis of the encoded primary structure. The gene ends immediately at the initiator codon of the *sopl* gene which encodes the sensory rhodopsin I apoprotein. Putative promotor elements are located in an AT-rich region upstream of the gene. Comparison of the translated nucleotide sequence with N-terminal sequence of the purified protein shows the protein is synthesized without a processed leader peptide and the N-terminal methionine is removed in the mature protein. The deduced protein sequence predicts two transmembrane helices near the N-terminal which would anchor the protein to the membrane. Beyond this hydrophobic region of 46 residues, the remainder of the protein (535 amino acid residues total) is hydrophilic. The C-terminal 270 residues contain a region homologous to the signalling domains of eubacterial transducers (e.g. *Escherichia coli* Tsr protein), flanked by two regions homologous to the methylation domains of the transducer family. The predicted protein structure differs from that of *E. coli* Tsr in that it does not have an extramembraneous receptor binding domain, but instead has a more extended cytoplasmic region.

DISTRIBUTION STATEMENT A

Approved for public release
Distribution Unlimited

92-25868



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SIGNIFICANCE: These results (1) extend the eubacterial transducer family to the archaeobacteria; and (2) substantiate the proposal that the methylated membrane protein functions as a signal transducing relay between SR-I and cytoplasmic sensory pathway components.

WORKPLAN (next 12 months): The next objective is to express MPP-I in the presence and absence of SR-I in *H. halobium*. Transformants will be studied for SR-I spectroscopic properties, MPP-I methylation, and SR-I-mediated phototaxis *in vivo*. Our preliminary results from SR-I expression in the absence of MPP-I indicate MPP-I influences the process of reprotonation of the Schiff base, an essential reaction in the transition of the SR-I attractant signaling conformation to the pre-stimulus state. The expression vector is based on an expression plasmid developed by Krebs and Khorana to which has been added the mevinolin resistance marker from Doolittle's laboratory.

Two additional clones hybridizing to the MPP-I probes were isolated during cloning of the MPP-I gene. These putative transducer genes will be sequenced and mapped and examined for function as chemotaxis or phototaxis (SR-II) transducers.

PUBLICATIONS (last 12 months):

1. Yan, B., Cline, S.W., Doolittle, W.F., and Spudich, J.L. (1992) Transformation of a BOP⁻HOP⁻SOP-I⁻SOP-II⁻ *Halobacterium halobium* mutant to BOP⁺: Effects of bacteriorhodopsin photoactivation on cellular proton fluxes and swimming behavior. Photochem. Photobiol. *in press*.
2. Spudich, J.L., and Bogomolni, R.A. (1992) Sensory rhodopsin I: Receptor activation and signal relay. Biomemb. and Bioenerg. **24**:193-199.
3. Olson, K., Deval, P., and Spudich, J.L. (1992) Absorption and photochemistry of sensory rhodopsin - I : pH effects. Photochem. Photobiol. *in press*.
4. Takahashi, T., Yan, B., Johnson, R., and Spudich, J.L. (1992) Sensitivity increase in the photophobic response of *Halobacterium halobium* reconstituted with retinal analogs: a novel interpretation for the fluence-response relationship and a kinetic model. Photochem. Photobiol. *in press*.
5. Yao, V. and Spudich, J.L. (1992) Primary structure of an archaeobacterial transducer, a methyl-accepting protein associated with sensory rhodopsin I. Proc. Natl. Acad. Sci. USA, *submitted*.

Related papers in this period:

1. Khan, S., Amoyaw, K., Spudich, J. L., Reid, G. P., and Trentham, D. R. (1992) Bacterial chemoreceptor signalling probed by flash photorelease of a caged serine. Biophysical J. **62**:67-68.
2. Lawson, M. A., Zacks, D. N., Derguini, F., Nakanishi, K., and Spudich, J. L. (1991) Retinal analog restoration of photophobic responses in a blind *Chlamydomonas reinhardtii* mutant: Evidence for an archaeobacterial-like chromophore in a eukaryotic rhodopsin. Biophys. J. **60**:1490-1498.

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Availability Codes	
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ANNUAL REPORT QUESTIONNAIRE
(for ONR use only)

Principal Investigator Name: John Lee Spudich
Institution: University of Texas Medical School-Houston
Project Title: Role of Protein Methylation in Halobacterium halobium
Phototaxis

Number of ONR supported

Papers published in refereed journals: 5

Papers or reports in non-refereed publications: 0

Books or book chapters published: 2 (1 book edited, 1 book chapter published)

Number of ONR supported patents/inventions
Filed: 0

Granted: _____ Patent name and number: _____

Number of presentations: Total ONR Project

Invited: 3

Contributed: 3 3 3

Trainee Data (only for those receiving full or partial ONR support):

	TOTAL	FEMALE	MINORITY	NON-US CITIZEN
No. Grad. Students:	1	1		
No. Postdoctorals:				
No. Undergraduates:				

AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP (please describe):

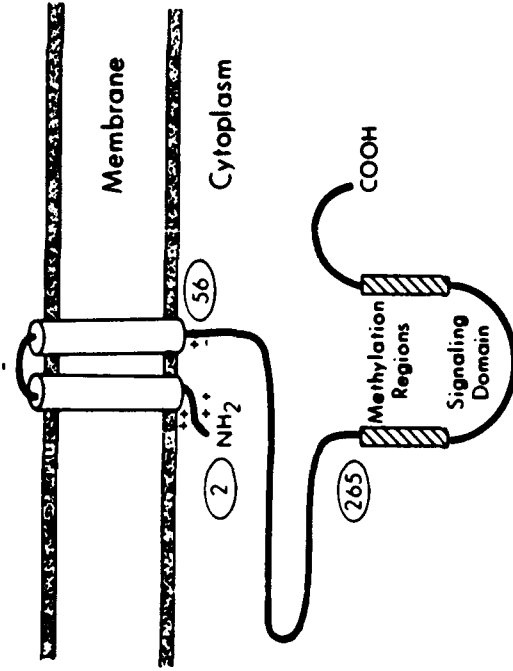
Dr. Karl Olson, post-doctoral researcher in P.I.'s research group, won an American Cancer Society Fellowship

Equipment purchased on grant (number and description of items costing >\$1,500):

1. Hoefer Scientific Minifluorometer	\$1867
2. MJ Research PCR thermocycler	\$2065

SR-I TRANSDUCER (MPP-I)

J.L. Spudich, University of Texas
at Houston; 1992



Objectives

- Sensory Rhodopsin I (SR-I) Signal Transduction
- Purify the proposed transducer (methyl-accepting protein) associated with SR-I
- Clone, map, and sequence the transducer gene

Accomplishments

- The protein isolated and tryptic peptides sequenced
- Gene cloned and predicted primary structure analyzed

Significance

- The first archaeobacterial transducer
- Homology with eubacterial transducers substantiates proposal that the protein relays signals from SR-I